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## Can protein functionalities be enhanced by high-pressure homogenization? – A study on functional properties of lupin proteins

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### Abstract

Many common foods like salad dressings or milk products are processed by high-pressure homogenization. Compared to conventional treatments, dynamic high pressures have been found to induce significant changes in the protein layer at the interface of these emulsions. But until now the influence of high-pressure homogenization on the functionality of proteins itself has only been scarcely studied. The aim of this study was to characterize the influences of high-pressure homogenization on the functional properties of lupin proteins of different lupin species because these proteins exhibit good functionality and can be applied as natural emulsifiers in food systems. Especially, the influences on the emulsifying and gelling properties of lupin proteins were studied. The gel strength and the emulsifying capacities of *L. albus* proteins were increased significantly after high-pressure homogenization with pressures up to 150 MPa at 35 and 60°C, respectively. The protein solubilities were only barely influenced by homogenization. Completely different results were obtained for *L. angustifolius* proteins. The emulsifying properties could not be enhanced by high pressure homogenization, which might be due to the presence of different protein fractions in protein isolates of these two species. These results imply that high-pressure homogenization has interesting potential for enhancing functional properties of lupin proteins of *L. albus* L., while the functional properties of *L. angustifolius* proteins after homogenization remained similar. High-pressure homogenization is therefore likely to be a tool for enhancing protein functionality, especially for proteins having inferior functionality due to heat treatment or prior processing.

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### 1. Introduction

High-pressure homogenization (with pressures up to 350 MPa) is a common process to form fine and stable emulsions in food products, pharmaceuticals, and cosmetics. In general food emulsions are formed

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by two immiscible liquids like oil and water that are emulsified by homogenization and stabilized by amphiphilic macromolecules (mainly proteins) or low molecular surfactants, which are adsorbed at the air-water interface. The mechanisms of stabilization and destabilization of food emulsions are well known and have been reported previously [1,2]. The influences of high pressure treatments on the structure of the interfacial layer and conformational changes of proteins have been studied widely using high hydrostatic pressure [3-6]. In contrast to hydrostatic pressure treatments, dynamic pressure during high-pressure homogenization lasts for very short time ( $\sim 10^{-4}$  s) and several effects like shear stress, turbulence, cavitation and temperature rise occur simultaneously [7]. Therefore, the effects of high hydrostatic pressure treatments and high-pressure homogenization on protein conformation and protein functionality can not be compared. Only few studies on the influences of dynamic high pressures on the functional properties of globular proteins are available, which report that functionality can be enhanced up to pressures of 150 to 200 MPa [7,8,9].

The present study was performed using lupin proteins as they are promising for the application in emulsified foods. Generally, lupin proteins exhibit good protein solubilities, good emulsifying and moderate gelation properties compared to soy proteins [10,11].

Therefore, the aim of the present study was to characterize the influences of high-pressure homogenization on the functional properties of lupin proteins of two different species (*L. albus* L. and *L. angustifolius* L.). Additionally, the particle sizes and protein patterns after high-pressure homogenization were analyzed using laser diffraction and SDS-PAGE, respectively.

## 2. Materials and Methods

Protein isolates from different lupin species (*L. albus* L. and *L. angustifolius* L.) were produced at pilot-scale according to the process described by Wäsche *et al.* [11]. Aliquots of solutions containing 10% (w/w) of lupin protein (pH 7.0) were homogenized at pressures of 0 to 150 MPa at inlet-temperatures of 35°C and 60°C, respectively, using the APV-2000 high-pressure homogenizer (SPX APV, USA).

After lyophilization the functional properties of the protein powders were determined. The protein solubility was analyzed in duplicate according to the method of Morr *et al.* (1985) and corresponded to the dissolved protein fraction relative to the protein content of the starting sample [12]. Protein solubilities of the homogenized protein powders were obtained by mixing an aliquot of 1 g of protein samples in 50 mL 0.1 mol L<sup>-1</sup> sodium chloride solution at pH 7.0 at room temperature for 60 min. The non-dissolved fractions were separated by centrifugation at 20000 g for 15 min. The protein content in the supernatant was determined by nitrogen analysis as described above.

The emulsifying capacities (EC) of the lupin samples were determined in duplicate according to the method described by Bader *et al.* and Wäsche *et al.* [10,11] using an one litre reactor equipped with a stirrer and an UltraTurrax (IKA-Werke GmbH & Co. KG, Staufen, Germany). 1 g of sample was dissolved in 99 g demineralized water at 18°C, homogenized using the UltraTurrax and 125 mL corn oil (Mazola®, Unilever Deutschland GmbH, Hamburg, Germany) was added to the protein solution. Additional oil was added by automatic titration with a Titrino 702 SM (Metrohm GmbH & Co. KG, Herisau, Switzerland) at a constant rate of 10 mL per min until phase inversion of the emulsion determined by conductivity breakdown. The volume of oil needed for phase inversion was used to calculate the EC (mL oil per g protein isolate).

In order to evaluate the gel strength of the protein isolates after high-pressure homogenization, solutions containing 15% (w/w) of lupin protein isolate were prepared under constant stirring for 30 min using demineralized water. The pH of these solutions was adjusted to pH 7.0 with 0.1 mol L<sup>-1</sup> sodium hydroxide, if required, and 0.5% (w/w) of sodium chloride was added. The protein solutions were conveyed to Bloom® glasses directly after preparation. To induce gelation the protein solutions were heated in a water bath at 95°C for 1 h. Subsequently, the coagulated solutions were cooled down and

stored at 1°C for 12 h. The gel strength (maximum penetration force in N cm<sup>-2</sup>) was determined at 5°C using the Texture Analyzer TA.XTPlus.

Additionally, the particle sizes of the solutions after homogenization were studied using the MasterSizer<sup>®</sup> S (Malvern Instruments GmbH, Germany). The protein solutions were diluted to obtain shadowing effects in the range of 14 to 21% using 0.01 mol L<sup>-1</sup> sodium phosphate buffer at pH 7 to prevent pH shifts during the measurements.

The protein patterns of the lupin protein isolates were analyzed by SDS-PAGE which was carried out at least in triplicate using the vertical gel unit Hoefer SE 600 Ruby (Amersham Biosciences, Germany). The samples were prepared according to the method described by Magni et al. [13]. In order to determine the molecular weights of the protein fractions a molecular weight standard from 10 kDa to 250 kDa (Precision Plus Protein Kaleidoscope<sup>™</sup> Standard, Bio-Rad Laboratories GmbH, Germany) was used and added on at least two lanes on the gel. The gels were stained using Coomassie Blue R 250, scanned in color and the molecular weight of each band was related to the molecular weight standard (Precision Plus Protein Kaleidoscope<sup>™</sup> Standard) using the Image Quant TL Software (Amersham Biosciences, Germany).

### 3. Results and Discussion

The aim of the present study was to characterize the influences of high-pressure homogenization on protein solubilities, emulsifying capacities, and gelation of lupin proteins of different lupin species (*L. albus* L. and *L. angustifolius* L.).

Homogenization of lupin protein isolates of *L. albus* L. and *L. angustifolius* L. were carried out at pressures of 0 (without applied pressure) to 150 MPa and at inlet-temperatures of 35°C and 60°C, respectively. At the outlet the temperature of the protein solutions increased by about 10 to 15°C directly depending on the homogenization pressure. The maximum outlet-temperature was 50°C for the first experimental series at 35°C, while the maximum outlet-temperature of the second experimental series at 60°C was 72°C. The temperature increase found in the present study was slightly lower, but still comparable to that found by Bouaouina et al. [7].

#### 3.1. Effect of high-pressure homogenization on protein solubility

After high-pressure homogenization, the protein solubilities of *L. albus* L. proteins were only slightly increased from 67% at 0 MPa to 73% at 150 MPa at 35°C inlet temperature. At the inlet-temperature of 60°C the protein solubility increased to 78% at 150 MPa. Overall, this slight increase in protein solubility might be due to the better dispersion or hydration of lupin protein isolates for *L. albus* L. For *L. angustifolius* proteins the protein solubility was similar after high-pressure homogenization compared to the untreated sample, which is in agreement to the results reported by other researchers [7,9,14].

#### 3.2. Effect of high-pressure homogenization on emulsifying capacities

In addition to the protein solubilities, the influence of high-pressure homogenization on the emulsifying properties of lupin protein isolates of *L. albus* L. and *L. angustifolius* L. were investigated. The ECs depending on the homogenization pressures of the protein isolates of *L. albus* L. and *L. angustifolius* L. at inlet-temperatures of 35°C are shown in Figure 1.

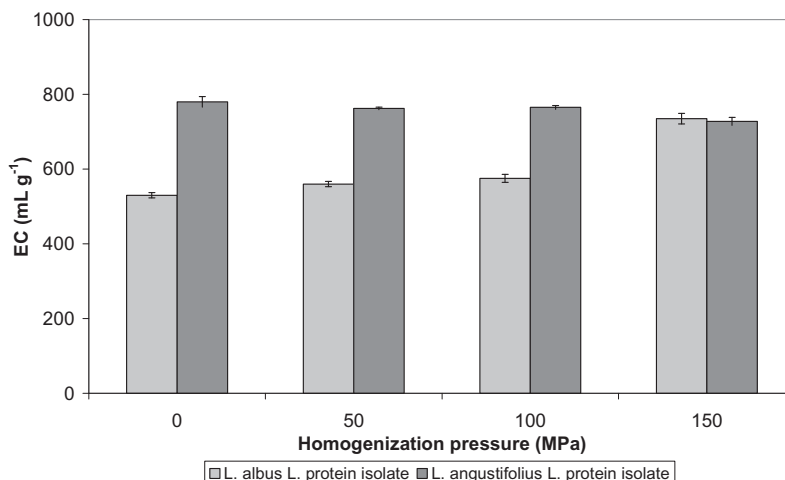


Fig. 1. Influences of homogenization pressure on the emulsifying capacities of lupin protein isolates (*L. albus* L. and *L. angustifolius* L.) at inlet-temperatures of 35°C (error bars represent standard deviation)

The EC of the untreated lupin protein isolates revealed values of 530 mL oil g<sup>-1</sup> for *L. albus* L. and 780 mL oil g<sup>-1</sup> for *L. angustifolius* L., respectively. Homogenization at pressures of 50 and 100 MPa increased the emulsifying capacities of *L. albus* L. proteins only slightly to 580 mL g<sup>-1</sup>, while at a pressure of 150 MPa the EC was significantly increased to 770 mL oil g<sup>-1</sup>, which is a value similar to the EC of the protein isolate of *L. angustifolius* L. Completely different results were obtained for protein isolates of *L. angustifolius* L. Whereas the untreated isolate had already an emulsifying capacity of 780 mL oil g<sup>-1</sup>, no further enhancement could be achieved by high-pressure homogenization at pressures up to 150 MPa. These differences might be attributed to variations in protein fractions present in the isolates of *L. albus* L. and *L. angustifolius* L. as shown in Figure 1. Treatments at the inlet-temperature of 60°C had no significant influence on the emulsifying properties of the protein isolate of *L. angustifolius* L. as well. However, for *L. albus* L. proteins the emulsifying capacity was increased to 715 mL g<sup>-1</sup> even for the untreated sample. At a homogenization pressure of 150 MPa the EC increased to 830 mL g<sup>-1</sup>. These results reveal that the temperature increase also has some effect on the EC of *L. albus* L. proteins.

The results for the *L. albus* L. protein isolate are in good agreement to the results previously obtained for high-pressure homogenization of whey protein isolates [7,9,15]. These researchers reported that high-pressure homogenization increased the surface activity of whey proteins, which may lead to advanced emulsifying properties by improving the efficiency to form interfacial layers [7,9,15]. Similar results were obtained by Flourey *et al.* who showed that the emulsifying properties of soy proteins can be enhanced by high-pressure homogenization from 20 to 200 MPa [8].

### 3.3. Effect of high-pressure homogenization on gel strength

In addition to the emulsifying properties, the gel strength of the homogenized protein samples was determined as described above. No gels were obtained for the untreated and treated lupin protein isolates of *L. angustifolius* L. at 15% (w/w) and therefore, no gel strength could be determined for these isolates. In contrast to these results, *L. albus* L. protein isolates formed protein gels at a concentration of 15% (w/w). The gel strength obtained for the untreated sample was 0.15 N cm<sup>-2</sup>, which represents a weak gel network and the gel strength was enhanced by high-pressure homogenization as shown in Figure 2. At both inlet-temperatures (35°C and 60°C) higher gel strengths were obtained for increasing

homogenization pressures of up to  $0.5 \text{ N cm}^{-2}$  ( $35^\circ\text{C}$ ) and  $0.6 \text{ N cm}^{-2}$  ( $60^\circ\text{C}$ ) after homogenization at 150 MPa. Thus, the gel strength of *L. albus* L. lupin protein isolate was directly influenced by high-pressure homogenization and increased about a factor of 4 after high-pressure homogenization at 150 MPa. An effect of the inlet-temperature on the gel strength was detected at 50 and 150 MPa, while at 100 MPa no significant differences occurred. Altogether, the gel network became firmer and the coagulated gels exhibited better cohesion compared to the untreated samples. Similar results were reported for whey protein isolates [9,14]. Increasing homogenization pressures lead to the formation of aggregates. This aggregation might be related to hydrophobic interactions which seem to result from ascending surface hydrophobicity as reported previously [7,9,14].

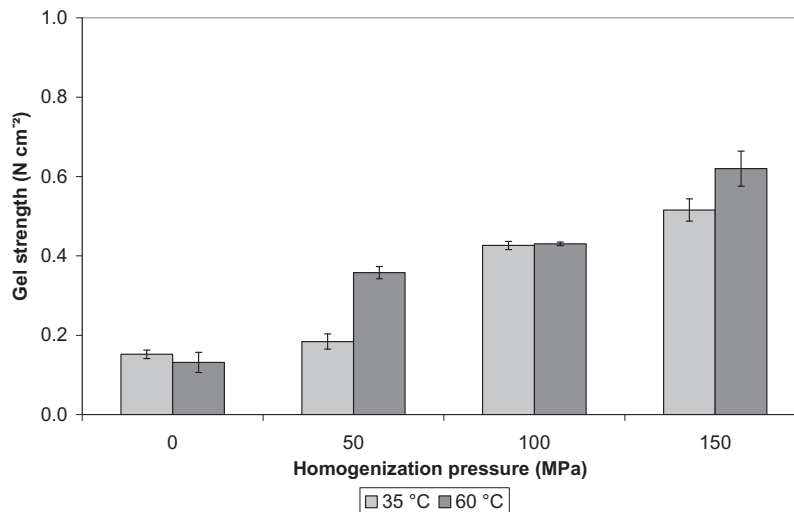


Fig. 2. Gel strength of *L. albus* L. proteins as a function of homogenization pressure and inlet-temperature (error bars represent standard deviation)

### 3.4. Effect of high-pressure homogenization on particle sizes

The particle size distributions of lupin protein solutions after homogenization at different pressures were analyzed at  $35^\circ\text{C}$  (Figure 3).

The distribution of the untreated solution is unimodal with protein aggregates bigger than  $1 \mu\text{m}$ . Increasing the homogenization pressure to 50 MPa shifted the particle size distribution towards smaller aggregates of 0.1 to  $10 \mu\text{m}$ . At 100 MPa the particle size distribution was comparable to that at 50 MPa. In contrast to these results, at a homogenization pressure of 150 MPa the formation of bigger aggregates with sizes about 10 to  $100 \mu\text{m}$  was promoted (Figure 3). These are as big as the aggregates present in the untreated lupin protein solution. Contradictory results were found for whey protein isolates after high-pressure homogenization by Bouaouina et al. [7]. The particle size distribution was similar for untreated whey protein isolate, while with increasing homogenization pressure the particle sizes decreased. No aggregation was found in that study.

However, these results are in good accordance with the results found in the present work. Lupin protein isolates (*L. albus* L.) exhibited higher emulsifying capacities and higher gel strength after high-pressure homogenization up to 150 MPa. One reason might be that the surface hydrophobicity of lupin proteins increased with increasing pressure, which can be attributed to a rising exposure of hydrophobic sites. Thus, the aggregation of lupin proteins at 150 MPa might be ascribed to hydrophobic interactions.

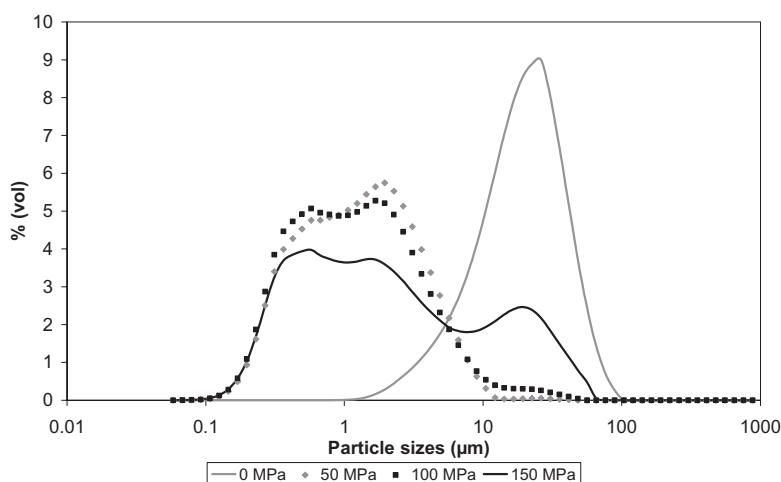


Fig. 3. Particle sizes of lupin protein solutions (*L. albus* L.) homogenized at different pressures at 35°C

### 3.5. Protein patterns of *L. albus* L. and *L. angustifolius* L. protein isolates

Additionally, the molecular weights of protein fractions present in both lupin protein isolates (*L. angustifolius* L. and *L. albus* L.) are shown in Figure 4.

The SDS-PAGE of lupin protein isolates revealed 22 bands for *L. angustifolius* L. and 19 bands for *L. albus* L., respectively. *L. angustifolius* L. exhibited protein fractions with molecular weights of 71 to 88 kDa, which were not present in *L. albus* L. Further differences were obtained in the range of 25 to 30 kDa. Therefore, the functional properties itself as well as the ability to enhance functional properties using high-pressure homogenization seem to be related to different protein patterns. However, possible mechanisms for enhancing protein functionalities have not been proposed until now.

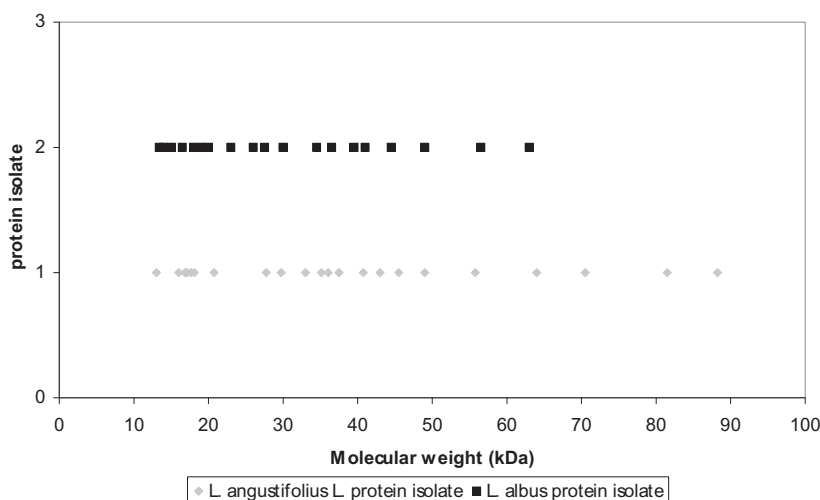


Fig. 4. Molecular weights of protein fractions present in *L. angustifolius* L. (1) and *L. albus* L. (2) protein isolate

#### 4. Conclusions

High-pressure homogenization showed interesting potential for enhancing functional properties of lupin proteins of *L. albus* L., while the functional properties of *L. angustifolius* proteins remained similar after homogenization. The dynamic high-pressure treatment induced disruption of protein aggregates for *L. albus* L. up to 100 MPa with only slight effect on protein solubility, whereas at 150 MPa re-agglomeration of protein particles occurred. High-pressure homogenization is therefore likely to be a tool for enhancing protein functionality, especially for proteins having inferior functionality due to heat treatment or prior processing. Additionally, the mechanisms of improving functional properties of proteins are not fully understood until now. Therefore, research activities should be directed towards the clarification of mechanisms and the influences of different protein fractions on functional properties.

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